

Treatment of Textile Wastewater by White-rot Fungi: Still a Far Away Reality?

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Abstract

The textile industry generates a huge volume of highly polluted effluents. The discharge of such effluents without an appropriate treatment is an issue of serious concern due to their aesthetic and toxic impacts on receiving waters. The traditional technologies for wastewater treatment are inefficient or costly in the treatment of textile effluents. This has impelled the search for new technologies to substitute or complement the existing ones. In this regard, white-rot fungi represent an eco-friendly and less expensive alternative for the treatment of such effluents. The present article reviews the decolouration of real wastewater by white-rot fungi up today.

Keywords

Decolouration; Ligninolytic Enzymes; Textile Wastewater; White-rot Fungi

Introduction

The textile industry generates large volumes of effluents with high concentration of dyes and salts, high biochemical oxygen demand (BOD), high chemical oxygen demand (COD), toxicity and usually also heavy metals (Rybicki et al., 2004). The discharge of textile effluents into the environment without appropriate treatment poses a serious threat for the ecosystem. The composition of the effluents generated from the textile industry is extremely variable and complex which makes their treatment particularly difficult. The traditional technologies for wastewater treatment include various physical and chemical

processes (primary treatments) coupled with a secondary biological treatment performed by activated sludge. These methods are usually ineffective for dye removal and a tertiary treatment is necessary such as advanced oxidation processes (AOPs). However, AOPs are costly and not always remove the toxicity (Vanhulle et al., 2008). The use of bacteria for dye removal may result in the formation of colourless dead-end aromatic amines, which are generally more toxic than the parent compounds (Banat et al., 1996). Hence, it is necessary to find new approaches to treat textile wastewater efficiently.

White-rot fungi are by far the only microorganisms able to degrade the whole wood components (i.e. lignin, cellulose and hemicellulose). This property is due to the secretion of extracellular non-specific ligninolytic enzymes, which are able to degrade the bulky recalcitrant polymer lignin. The main extracellular enzymes participating in lignin degradation are lignin peroxidase (ligninase, LiP, EC 1.11.1.14), manganesedependent peroxidase (MnP, EC 1.11.1.13), laccase (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) and versatile peroxidase (VP, EC 1.11.1.16). The same mechanism that gives these fungi the ability to degrade lignin allows them degrading a wide range of compounds with structures similar to lignin such as synthetic dyes.

The major drawback of the enzymatic treatment is the inactivation of enzymes in the conditions normally found in textile wastewater. Furthermore, the high cost of enzyme production limits its application to an

industrial scale. However, with a whole cell culture the enzymes can be continually replenished. Additionally, in industrial operations, immobilised microbial cell systems provide the following advantages over free cells: easy separation of cells from the liquid medium, thus, simplifying downstream processing, protection from shear damage, reduction in protease activity and the possibility to operate in continuous mode. In addition, immobilised cultures are more resilient to environmental perturbations such as pH or exposure to toxic chemical concentrations than free cultures (Shin et al., 2002).

Various publications have shown the ability of white-rot fungi to degrade synthetic dyes (Kaushik and Malik, 2009 and references therein). However, publications dealing with the treatment of real textile wastewater by white-rot fungi are scarce. The present article reviews the decolouration of real wastewater by white-rot fungi up today. To the best of the author's knowledge this topic has not been reviewed before this article.

Wastewater from the Textile Industry

The textile industry generates a huge volume of highly polluted effluents. Colour is noticeable at a dye concentration of 1 mg/L and an average concentration of 300 mg/L has been reported in effluents from textile manufacturing processes. During the dyeing processes, 2-60% of the initial used dyes are lost in the effluents (Hessel et al., 2007). The presence of dyes in water bodies reduces sunlight penetration into deeper layers diminishing photosynthetic activities of aquatic flora and decreasing the amount of dissolved oxygen in the water, which causes adverse effects on aquatic flora and fauna (Nilsson et al., 1993). Furthermore, dyecontaining effluents increase BOD of the contaminated water (Annuar et al., 2002). Also, adverse impact in terms of COD, toxicity and carcinogenicity makes textile industry one of the most polluting ones worldwide (Vandevivere et al 1998). Table I shows the general characteristics of wastewater from the textile industry (Puscas et al., 2003).

White-rot Fungi

White-rot fungi have attracted considerable attention since the 1980s, when the composition of ligninolytic

TABLE I GENERAL CHARACTERISTICS OF TEXTILE WASTEWATER (Puscas et al., 2003)

pH	4-12 (4.5 knitted wool, 11 cotton)
COD	150-250 mg/L
BOD	80-500mg/L
COD/BOD	3-5
Colour	500-2000 Pt-Co units
Suspended matter	30-400 mg/L; sometimes 1000 mg/L for cotton
Cr (VI)	1-4 mg/L
Sulphide	0-50 mg/L

systems was elucidated. They constitute a diverse ecophysiological group comprising mostly basidiomycetous fungi and to a lesser extent litter-decomposing fungi (Wesenberg et al., 2003) which are able to degrade the heterogeneous, complex and stable polymer lignin. The name white-rot derives from the white appearance of the wood when is attacked by these fungi due to lignin removal (Pointing, 2001). The ability to degrade lignin of white-rot fungi is correlated to their capacity to synthesise an extracellular non-specific enzymatic complex. This enzymatic complex consists mainly of lignin peroxidase (ligninase, LiP, EC 1.11.1.14), manganesedependent peroxidase (MnP, EC 1.11.1.13), laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) and versatile peroxidase (VP, EC 1.11.1.16). In addition, the following enzymes are associated with lignin-degrading enzymes in lignin breakdown but they are unable to degrade lignin alone: glyoxal oxidase (EC 1.2.3.5), superoxide dismutase (EC 1.15.1.1), glucose oxidase (EC 1.1.3.4), aryl alcohol oxidase (EC 1.1.3.7) and cellobiose dehydrogenase (EC 1.1.99.18). They produce the hydrogen peroxide required by peroxidases (LiP and MnP) or serve to link lignocelluloses degradation pathways (Leonowicz et al 2001). Table 2 summarises the ligninolytic enzymes and their substrates and reactions (Hatakka, 2001). The nonspecific nature of the ligninolytic enzymes allows them degrading a wide variety of persistent

environmental pollutants (Barr and Aust, 1994), including synthetic dyes (Wesenberg et al., 2003; Robinson et al 2001).

In addition to their extracellular ligninolytic system, whiterot fungi have also an endocellular system involving cytochrome P-450 monooxygenase-epoxide hydrolase, which is supposed to cooperate with the ligninolytic system in the general mechanism of xenobiotic degradation (Bezalel et al., 1996; van den Brink et al., 1998).

Decolouration of Textile Wastewater by White-rot Fungi

Most research on dye decolouration has been performed with individual dyes or with simulated textile wastewater and there are few articles reporting the decolouration of real textile wastewater by white-rot fungi. Table 3 reports the use of different white-rot fungi to treat wastewater from textile industries.

Wesenberg et al (2002) reported the decolouration of wastewater from a textile dye-producing plant by the agaric white-rot fungus *Clitocybula dusenii*. They found that operating in the optimal conditions up to 87% of the dyes of a 4-fold diluted effluent were decolourised after 20 days of incubation. Also, ref. (Amaral et al., 2004) studied the decolouration of an effluent from a textile industry by *Trametes versicolor* and found that for a 7-fold diluted effluent a decolouration percentage of 40% was obtained which was much lower than that found for a synthetic effluent with the same colour (300 mg/L). A higher decolouration percentage (92%) was attained with a 42-fold diluted effluent (dye concentration 50 mg/L). Therefore, despite the authors of the above-mentioned papers stated that *C. dusenii* and *T. versicolor* were promising fungi for the decolouration of textile effluents dilution of the effluents was required. Also, ref. (Sangeeta et al., 2011) and ref. (Pakshirajan and Kheria, 2012) showed that effluent dilution with media containing carbon and other nutrients was required for high removal of both COD and colour by the model ligninolytic fungus *Phanerochaete chrysosporium*. In addition, ref. (Liu et al.,

2012) found that dilution of wastewater by 1/5 was required to avoid inhibition of fungal growth when *Pycnoporus* sp was cultured in wastewater from a textile industry. Likewise, ref. (Yildirim et al., 2012) reported that COD removal in wastewater from a textile plant by *Pleurotus djamar* was 80% when it was diluted by 1/5 with Sabouraud dextrose broth whereas it was much lower (47%) when it was diluted by 1/2.

Ref. (Ramsay and Goode, 2004) studied the decolouration of a carpet dye effluent by *T. versicolor* and found that the fungus decolourised the effluent by 95% after 10 h of incubation at flask scale. In addition, *T. versicolor* immobilised on jute twine in a rotating biological contacting bioreactor (working volume 2.5 L) was able to decolourise the carpet dye effluent for 4 successive batches. Ref. (Kamida et al., 2005) investigated the decolouration of a textile effluent by two strains of *Pleurotus sajor-caju* grown on sugarcane bagasse and found that both strains totally decolourised the effluent after 14 incubation days. Ref. (Nilsson et al., 2006) reported a decolouration percentage of about 70% for a textile wastewater by *Pleurotus flabellatus* grown on luffa sponge in a reactor (working volume 1.5 L) operating in continuous mode at a hydraulic retention time (HRT) of 26 h. In this case, wastewater was autoclaved, nutrients and extra carbon source were added and pH was adjusted to 3-4. Despite the advantages of using a natural support (cost reduction, induction of ligninolytic activities and reutilisation of agro-wastes), it might pose serious limitations to the process such as degradation of the support along the process and there is also the additional problem of the disposal of the spent dyed support.

Ref. (Srikanlayanukul et al., 2006) investigated the decolouration of wastewater from Batik dyeing process by *Coriolus versicolor* immobilised on 1.5-cm³ cubes of polyurethane foam (PUF) in a 10-L air-bubble bioreactor. They found that the fungus decolourised the effluent up to 80% and COD was reduced by 67% in 48 h. The authors did not mention whether dye adsorption on PUF cubes and/or fungal mycelium took place.

Blanquez et al. (2008) showed that *T. versicolor* was able to continuously decolourise a spent dyeing bath from a textile factory under non-sterilised conditions for 15 days in a 10-L air pulsed bioreactor, attaining colour reduction levels between 40 and 60%. Nutrients were added at the starting-up period (3 days) of the experiment and thereafter sterilised glucose was added. Therefore, although the authors stated that *T. versicolor* was able to treat successfully real industrial wastewater in continuous mode (HRT 48 h), the operation time was very short (15 days) and, in addition to this, bacterial contamination in the feeding tank was detected from day 10.

Gomez-Bertel et al. (2008) reported the decolouration of an effluent from a tannery industry by *P. cryosporium* immobilised in 1-cm³ cubes of polyurethane foam at flask scale. The immobilized viable biomass removed 97% of total Cr, 97% of Cr(III), 85% of Cr(VI), 12% of COD and 67% of colour in 10 days. They also found that the inactive fungal biomass was able to reduce the above-mentioned parameters although in lower proportion.

Asgher et al. (2009) showed the ability of *C. versicolor* to decolourise the textile effluents collected from 5 different textile industries. However, addition of starch (1% w/v) as a carbon source was required to obtain significant decolouration levels (84% in 3 days).

Anastasi et al. (2010) reported the decolouration of wastewater from a dyeing-textile factory by the white-rot fungi *Bjerkandera adusta* packed in a fixed-bed bioreactor. The fungus resulted effective during 4 cycles of decolouration, remaining active for a very long period (70 days) under non-sterile conditions and with no nutrient addition. Furthermore, the fungal treatment reduced considerably the COD and the toxicity of the effluent. In this case dilution of wastewater, addition of nutrients and control of chemical parameters were not required which showed the applicability of the developed system. The authors stated that large volumes of wastewater were treated; however their experiments were performed at laboratory scale. Later, they tested the capacity of the same fungus to degrade wastewater from a textile

industry after a secondary treatment (Anastasi et al., 2011). They found that the fungal treatment caused a decolouration of the effluent of 40% in 24 h, which was less efficient than that obtained by ozonation (63% in 15 min.). More recently, the same authors showed that fungal treatment, especially with the fungus *Trametes pubescens*, followed by activated sludge of wastewater from a cotton dyeing industry led to very good results in terms of decolouration (over 60%), COD reduction and toxicity removal (Anastasi et al., 2012). However, the scaleup in a 5-L moving-bed bioreactor (working volume 2 L) with *T. pubescens* immobilised on 2-cm³ cubes of PUF led to lower decolouration (30%). Here again, the authors claimed that large volumes of wastewater were treated despite their experiments were conducted at laboratory scale.

Osorio Echevarria et al. (2012) reported the decolouration of wastewater from a textile industry by the anamorph R1 of the white-rot fungus *Bjerkandera* sp. Under sterile conditions the effluent was decolourised by 65% in 8 days and its toxicity was reduced by 58% whereas under non-sterile conditions the decolouration percentage was only 40% for the same time period. The authors stated that this lower decolouration value was likely due to the presence of contaminant microorganisms competing by the substrate.

Selvakumar et al. (2012) treated textile wastewater in a batch reactor with *Ganoderma lucidum*. Under optimised conditions (pH 6.6; temperature 26.5°C; agitation speed 200 rpm; dye wastewater concentration 1:2) a maximum decolouration of 81.4% and a COD reduction of 90.3% were found. Therefore, dilution and pH decreased of the original effluent were necessary for maximum decolouration and COD reduction.

Conclusions

Although several publications have shown the potential of white-rot fungi to treat real wastewater from the textile industry, most of them have been performed with some preconditioning of wastewater (dilution, pH adjustment, sterilisation, addition of nutrients). Also, there are scarce studies at bioreactor scale operating in continuous mode and under

non-sterilised conditions. Therefore, nowadays the application of such fungi at industrial scale is still a technical challenge. In addition, detailed characterisation of the intermediates and metabolites

produced during biodegradation must be done in order to assess the true technical potential of white-rot fungi. Moreover, toxicity studies should be performed to ensure the safety of the decolourised wastewater.

TABLE II LIGNINOLYTIC ENZYMES AND THEIR MAIN REACTIONS (AFTER Hatakka, 2001)

Enzyme and abbreviation	Cofactor	Substrate, mediator	Reaction
Lignin peroxidase, LiP	H ₂ O ₂	Veratryl alcohol	Aromatic ring oxidised to cation radical
Manganese peroxidase, MnP	H ₂ O ₂	Mn, organic acids as chelators, thiols, unsaturated fatty acids	Mn(II) oxidised to Mn(III); chelated Mn(III) oxidises phenolic compounds to phenoxyl radicals; other reactions in the presence of additional compounds
Versatile peroxidase, VP	H ₂ O ₂	Mn, veratryl alcohol, compounds similar to LiP and MnP	Mn(II) oxidised to Mn(III), oxidation of phenolic and non-phenolic compounds and dyes
Laccase	O ₂	Phenols, mediators, e.g., hydroxybenzotriazole or ABTS	Phenols are oxidised to phenoxyl radicals; other reactions in the presence of mediators
Glyoxal oxidase, GLOX		Glyoxal, methyl glyoxal	Glyoxal oxidised to glyoxal acid; H ₂ O ₂ production
Aryl alcohol oxidase, AAO		Aromatic alcohols (anisyl, veratryl alcohol)	Aromatic alcohols oxidised to aldehydes; H ₂ O ₂ production
Other H ₂ O ₂ -producing enzymes		Many organic compounds	O ₂ reduced to H ₂ O ₂

TABLE III DECOLOURATION OF REAL WASTEWATER BY WHITE-ROT FUNGI

Fungus	Source of wastewater	Reference
<i>Clitocybula duseinii</i> DSM 11238	Textile dye-producing plant	van den Brik (1998)
<i>Trametes versicolor</i>	Textile industry (Brazil)	Wesenberg et al. (2002)
<i>T. versicolor</i> ATCC 20869	Carpet dye effluent	Amaral et al. (2004)
<i>Pleurotus sajor-caju</i> CCB 020;	Textile industry (Brazil)	Ramsay and Goode (2004)
<i>P. sajor-caju</i> PSC 94/03		
<i>Pleurotus flabellatus</i>	Textile industry (Tanzania)	Kamida et al. (2005)
<i>Coriolus versicolor</i> RC3	Batik dyeing factory (Thailand)	Nilsson et al. (2006)
<i>T. versicolor</i> ATCC 42530	Textile industry (Spain)	Srikanlayanukul et al. (2006)
<i>P. cryosporium</i>	Tannery industry (Colombia)	Blaquez et al. (2008)
<i>C. versicolor</i> IBL-04	Arzoo Textiles, Crescent Textiles, Itmad Textiles, Megna Textiles and Ayesha Textiles (Pakistan)	Gomez-Bertel et al. (2008)
<i>Bjerkandera adusta</i> MUT 2295	Tintoria Pollone, Biella (Italy)	Asgher et al. (2009)
<i>B. adusta</i> MUT 3060	EURO D srl (Italy)	Anastasi et al. (2010)
<i>Bjerkandera</i> sp. (anamorph R1)	Dyeing process <i>Pat Batch</i> from a textile factory (Colombia)	Anastasi et al. (2011)
<i>T. pubescens</i> MUT 2400	FelliColor SpA (Italy)	Sangeeta et al. (2011)
<i>Pycnoporus</i> sp. SYBC-L3	Textile industry (China)	Anastasi et al. (2012)
<i>Ganoderma lucidum</i> MTCC 1039	Textile industry (India)	Osorio Echavarria et al. (2012)

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